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Antioxidant activity of 2-Thioxo Imidazolidin-4-Ones in male rats exposed to oxidative stress by hydrogen peroxide in drinking water

Jiheel M. Ja
Department of Biology, College of Science, University of Wasit, Iraq
Email: mjiheel@uowasit.edu.iq

Samar Abdullaha Zayir
Department of Biology, College of Science, University of Wasit, Iraq
*Corresponding author email: samarzaeer@gmail.com

Sallal A. H. Abdullahab
Chemistry division, Applied Science department, University of Technology-Iraq
Email: sallal.a.abdullaha@uotechnology.edu.iq

Abstract---In the present study, a new Thioxoimidazolidine derivative had been preparedasligand, L=(3{(E)[(4bromophenyl) methyldenamino2sulfanylideneimidazolidin-4-one. This ligand was synthesized by the reaction of 4-bromobenzaldehyde with thiosemicarbazide, then, the product was reacted with chloroethylacetate in an ethanol medium. The final product was isolated and characterized by suitable physical measurements, FT-IR, UV-Vis spectrophotometer, GC-mass spectrophotometer, and 1H-NMR spectroscopy as well as elemental analysis (CHNS). for assessment of the antioxidant activity of this new ligand in vivo we used twenty-four mature albino male rats divided into three equal groups, the first group served as control and received a normal diet and drank from tap water, while the other groups received a normal diet and drinking of 0.5%hydrogen peroxide, third group dosage by 50mg/kg of Thioxoimidazolidine daily. The results showed a decrease in GSH Catalase and total antioxidant concentrations of animals of selected groups when treated with 0.5% v/v of hydrogen peroxide, while a significant elevation in serum GSH, Catalase, and total antioxidant concentrations in another group after treatment with Thioxoimidazolidine of ligand.

Keywords---oxidative stress, antioxidant, 2-Thioxo imidazolidine, catalase.

Introduction

A loss of equilibrium between the pro-oxidant and antioxidant is known as oxidative stress. (*Pisoschi et al.2021*). An imbalance between oxidation and reduction occurs in organisms when there are too many oxidants and too few antioxidants. Oxidative stress is caused by the antioxidant system's deficiency and is characterized by high quantities of reactive oxygen species, hydroxyl free radicals, and other compounds. (*Singh et al.2019*). Thus, oxidative stress can be classified according to its level of intensity as either physiological oxidative stress (eustress) or toxic oxidative stress, alternating biomolecules (distress): low exposure of cells and organisms is required for redox signaling, directed towards specific targets, whereas elevated exposure results in disruption of redox signaling and/or decay, addressing unspecific targets. (Sies.2018). Reactive oxygen species that are created as a result of oxidative processes bind to DNA, proteins, lipids, and other biological components, resulting in structural damage.

Therefore, oxidative stress is the term used to describe the oxidative damage brought on by reactive oxygen derivatives. (Taşbozan et al.2019). Free radicals are molecules with unpaired electrons in the outer orbitals that are highly reactive in the body and capable of oxidizing or occasionally reducing (donating an electron to) other atoms. (Adwas.2019) A substance known as an antioxidant can stop or delay the oxidation of macromolecules. By eliminating free radicals or preventing other oxidation events by becoming oxidized themselves, antioxidants' function is to slow or stop these chain reactions. (Duarte and Lunec.,2005) Compounds called antioxidants stop oxidation. Free radicals are created during the chemical process of oxidation, which can cause a cascade of events that harm living beings' cells. Thiols and ascorbic acid (vitamin C) are antioxidants that stop these processes. Plants and animals maintain extensive systems of intervening antioxidants to regulate the oxidative state, including endogenous antioxidants like glutathione and enzymes like catalase and superoxide, as well as ingested antioxidants like vitamins C and E. (Pawar.2016).

Antioxidants were employed to stop food from going rancid, browning, or oxidizing DNA, all of which have beneficial physiological impacts on people. Although more research is required to fully understand their physiological effects, a concerted effort to educate people about foods strong in natural antioxidants and the capability to identify the main synthetic antioxidants on food labels would be very advantageous. (Atta et al,.2017). Recently, there has been a rise in interest in synthetic antioxidants as a potential treatment**,** To stop fats from oxidizing, it is utilized in the pharmaceutical and cosmetic sectors. Piperidine derivatives, or imidazolidine derivatives, have a variety of pharmacological effects, including antitubercular effects. (Aridoss et al., 2008), HeLa cell cytotoxicity (Parthiban et al., 2011), antihypertensive (Watanuki et al., 2011), anticancer (Girgis, 2009), coronary vasodilation (Dabaeva et al., 2008), and antiarrhythmic properties (Abdel-Aziz et al.,2009). According to Czopek et al. (2010), derivatives of imidazolidine-2,4-dione have pharmacological activities like anxiolytic and antimalarial properties (Araujo et al., 2005), In order to synthesize piperidine with imidazolidine derivatives and test its anticoagulant and anticancer properties, these references will serve as the primary bases. (Ashraf et al., 2016). After 24 and 48 hours, respectively, the inhibitory concentrations (IC50) of 2-Thioxo

imidazolidin-4-ones (2-Thiohydantion) compound on MCF7 cells are 135 and 40 g/ml, respectively. (jiheel et al., 2020). Therefore, the current study was done to show how Imidazolidine derivati works to lessen the negative consequences of oxidative stress.

Materials and Methods

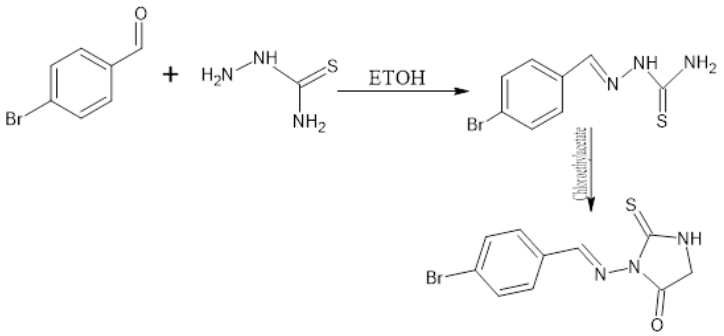
All chemical were of highest purity and were used as received.

Preparation of Hydrogen peroxide solution

Absolute hydrogen peroxide was utilized at a 50MN concentration and diluted to 0.5 percent with ordinary drinking water. Daily preparation of the solution resulted in its administration to test animals in specific bottles. There is a 60-day trial period.

Synthesis of Ligand

A solution of thiosemicarbazide (0.58gm, 0.0054 mol) was added to the solution of (1g, 0.0054 mol in 10mL of absolute ethanol) of 4-bromobenzaldehyde. The resulting mixture was reflexed for a few minutes and then drops of concentrated acetic acid were added. The mixture was heated under reflux for (6 h.), then the mixture was cooled and poured into crushed ice, and the resulting solid produced was filtered, dried, and recrystallized from ethanol. (MP = 224-226C^0). Scheme (1). (0.00168mol, 0.5g) of the previous product was dissolved in absolute ethanol, then (0.205g, 0.00168mol) of ethyl chloroacetate was added. This mixture was heated under reflux for (3h.), and the resulting solid product was filtered, dried, and recrystallized from ethanol, (MP =264-262C). Scheme (1).



Scheme 1. Synthesis of Ligand (L)

Design of the experiment

In this study, Twenty-four adult albino male rats were used, and the experiment lasted for sixty days from Date twenty-three of January until twenty-three March 2022. The animals were randomly divided into three equal groups. The first group returned to the control group, as it was given the necessary food and water, As for the second, and third groups, the study was conducted on them for two months.Second, the third group was given 0.5% hydrogen peroxide in drinking

water. The third group was dosage by orally 2-Thioxo Imidazolidin-4-Ones by Cavage Needle . after thirty and sixty days of the experimental period at 10 o'clock, the blood sample was drawn from each animal, synthesized by a mixture of ketamine in a dose of 90 mg/Kg B.W. and xylazine, 40 mg/Kg B.W. an intramuscular route injectable that is safe (jiheel ,2015). Following completion of laboratory testing, they were compared with animals in the control group, and after sixty days, the same processes were performed with blood samples placed in a centrifuge at a power of 3000 cycles/ After the centrifugation process, the serum layer was isolated from the rest of the blood components. The serum was withdrawn by a fine pipette and then placed in Abendroth tubes to conduct functional tests. After that, the serum samples were kept at a temperature of -20°C to preserve them from damage until use.

Results

FTIR spectrum of compound (A)

FTIR spectrum of compound (A), fig (3.1) showed two bands at (3433cm^{-1}) and (3284cm^{-1}) related to ν (NH_2) vibration, while a single band at (3163cm^{-1}) related to ν (NH) vibration, the spectrum also showed bands at (3012cm^{-1}) and (2802cm^{-1}) related to ν (C-H) aromatic and ν (C-H) aliphatic vibration respectively. Band at (1695cm^{-1}) belonged to vibration of Azomethine group ν (C=N) , finally band at (1080cm^{-1}) related to ν (C=S) vibration(Nakamoto,.1997)

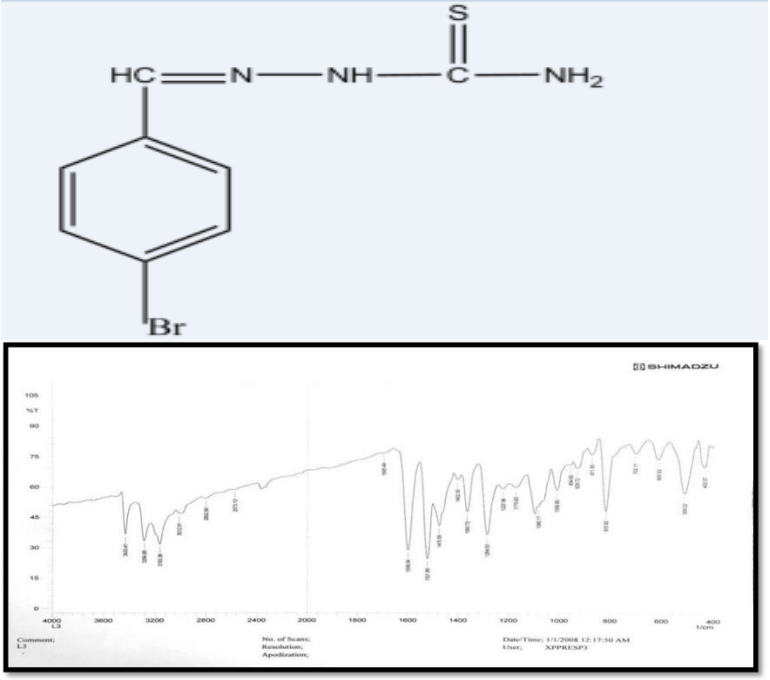


Figure 3-1. FTIR spectrum of compound (A)

UV spectrum of ligand (L)

The (U.V) spectrum of the synthesized ligands (L), Figure(3.5)in absolute ethanol showed three absorption bands, the, and band at first at (220nm, 454.55cm^{-1}) and (280 nm, 35714.29cm^{-1}) which assigned to (π→π*) and band at (310nm, 32258.06cm-1) due to(n→π*) (Silverstein, 1981).

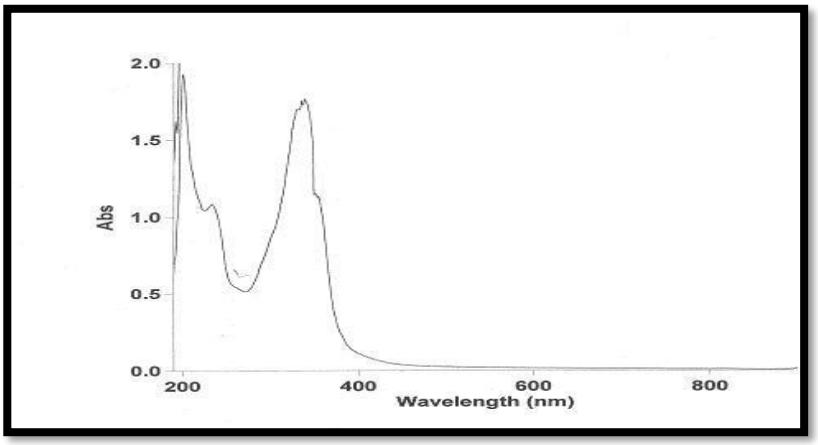


Figure 3-5. U.V spectrum of (L)

Serum GSH

Table (1) demonstrated that values of serum GSH concentrations of animals of the GII group that treatment with 0.5% hydrogen peroxide in drinking a significant reduction after thirty days and sixty days compared with a control group. while a significant elevation in serum GSH concentrations in the GIII group after treatment with 2-Thioxo Imidazolidin-4-Ones.

Serum Catalase

Table (2) showed serum Catalase concentrations of animals of the GII group that treatment with 0.5% hydrogen peroxide in drinking a significant reduction after thirty days and sixty days compared with a control group, while a significant elevation in serum Catalase concentrations in the GIII group after treatment with 2-Thioxo Imidazolidin-4-Ones after sixty days of experimental Periods means the antioxidant activity ability of extract in limited periods.

Serum total antioxidant

The results shown in table (3) after thirty and sixty days the serum antioxidant concentration levels at GII appeared to be a significant reduction when compared with the control group. The result of antioxidants of the GIII group after thirty and sixty days appeared a significant elevation when compared with GII and control group.

Table 1
Effects of 2-Thioxo Imidazolidin-4-Ones in male rats exposed to 0.5% hydrogen peroxide in drinking on GSH

GSH	30 Days				60 Days			
	Mean	±	SD		Mean	±	SD	
GI\Control	0.072	±	0.004	Aa	0.067	±	0.006	Ab
GII\H2O2	0.050	±	0.011	Ab	0.033	±	0.004	Bc
GIII\imo	0.077	±	0.007	A a	0.078	±	0.007	A a

Table 2
Effects of 2-Thioxo Imidazolidin-4-Ones in male rats exposed to 0.5% hydrogen peroxide in drinking on Catalase

Catalase	30 Days			60 Days		
	Mean	±	SD	Mean	±	SD
GI\Control	0.063	±	0.005 A b	0.066	±	0.003 A b
GII\H2O2	0.045	±	0.008 A c	0.037	±	0.006 Bc
GIII\imo	0.073	±	0.009 A a	0.075	±	0.008 A a

Table 3
Effects of 2-Thioxo Imidazolidin-4-Ones in male rats exposed to 0.5% hydrogen peroxide in drinking on Total antioxidant

Total antioxidant	30 Days			60 Days		
	Mean	±	SD	Mean	±	SD
GI\Control	0.061	±	0.007 A b	0.060	±	0.007 A b
GII\H2O2	0.040	±	0.006 A c	0.028	±	0.004 Bc
GIII\imo	0.073	±	0.009 B a	0.088	±	0.009 A a

Results and Discussion

Our study's findings indicate that animals in the GII group treated with 0.5 percent hydrogen peroxide in drinking water had significantly lower serum GSH concentrations. This may be because GSH is essential for oxidation and reduction reactions and hydrogen peroxide in drinking water works to release GSH into the blood and tissues. By blocking the pathway of GSH's conversion to pentose phosphate shunt, which determines the production of NADPH necessary for the activity of the glutathione reductase enzyme to restore GSH synthesis, Martin and his team demonstrated that the state of oxidative stress results in an increase in the oxidation of GSH to the disulfide form of GSSG (Martins et al,.1985). The cause of the reduction (*Clavel et al.,1985*). Giving them hydrogen peroxide in their water to drink resulted in the production of free radicals, and an increase in free radicals causes a decrease in the body's antioxidant levels, which causes damage to the body's numerous tissues. (*Dalle-Donne et al,.2006*).

Cells that are under oxidative stress tend to have weak antioxidant defense mechanisms. Free radical activity rises in many disease situations that lead to oxidative stress. High amounts of ROS in the mitochondria can lead to a free radical attack on the phospholipids in the membrane and depolarize the mitochondrial membrane. (*Zhou et al,.2009*). Following treatment with 2-Thioxo Imidazolidin-4-ones, antioxidant levels were markedly raised to (0.088), and it was noted that GSH levels increased to (0.078), and catalase levels increased to (0.075). *(Nafie et al.,2021)* Antioxidant levels were markedly raised. because the medication made apoptosis more controlled. The mitochondrial (intrinsic) or death receptor (extrinsic) pathways are typically used in apoptosis. Antioxidant levels increased because a lack of GSH and catalase is linked to mitochondrial malfunction and cell death. Chemically speaking, the presence of the attracted cyanic group (CH2CN) inserted on the (2-thioxoimidazolidin-4) in position 3 could be attributed to the antioxidant activity of (2-thioxoimidazolidin-4). This group (CH2CN) significantly affects the activity. Moreover, compound 6b's scavenger activity (4.9 g/mL, comparable to vitamin C) is increased by the presence of two amid groups in positions 3 to 4 on the thiohydantoine ring.(*Aichouchebouzroura et al.,2014*).

Conclusions

- In general, the present work describes the reaction of 4-bromobenzaldehyde with thiosemicarbazide to produce (2*E*)-2-[(4 bromophenyl) methylidene]hydrazine-1-carbothioamide; this product reacted with chloroethylacetate to form thioxoimidazolidine, the final products (L).
- These ligands were characterized by (UV-Vis, IR and 1HNMR).
- ligand have an anti-oxidative activity that revealed by an increase in serum GSH, Catalase and total antioxidants.

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